Plant Physiol. (1974) 53, 21–27

Translocation of Radioactive Carbon after the Application of ^{14}C-Alanine and $^{14}CO_2$ to Sunflower Leaves[1]

Received for publication January 26, 1973 and in revised form August 24, 1973

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ABSTRACT

^{14}C-(UL)-L-Alanine was applied to the surface of mature leaves at the second node of sunflower (*Helianthus annuus* L. cv Commander) plants, under illumination. The alanine was absorbed during a 4-hour period, and some of it was metabolized by the absorbing tissue. After a lag period of about 15 minutes from first application, distribution of ^{14}C through the plant proceeded in much the same pattern as when $^{14}CO_2$ is assimilated by similar leaves. Most, if not all, of the ^{14}C exported from the absorbing regions was in sucrose. Only minute amounts appeared in alanine or other amino acids in surrounding parts of the leaf blade or in the petiole, although these were strongly labeled in the tissue absorbing ^{14}C-alanine.

When $^{14}CO_2$ was supplied for 15 minutes to leaves of different ages, amino acids were lightly labeled in the leaf blade. Mature green leaves exported only sucrose. Yellowing leaves on 60-day-old plants exported a variety of substances including amino acids.

The mobilization of organic nitrogen in plants has been investigated much less extensively than the distribution of carbohydrate. It is generally assumed that older leaves supply organic metabolites, including nitrogen compounds, to younger growing organs (14, 19, 22, 27, 33). While there are many indications that organic nitrogen is exported from senescing leaves, there is little direct evidence for its translocation in the phloem from mature leaves at the stage when they are actively supplying carbohydrate to vegetative growing regions.

A few well attested examples demonstrate export of ^{14}C-labeled amino acids from mature leaves of legumes (4, 6, 17). Oghoghorie and Pate (19) showed that ^{15}N absorbed by leaves of the field pea moved freely to other parts of the plant and stated that documentation is forthcoming for the occurrence of amino acids in the phloem sap of petiole and stem in this plant. Pate and his associates (2, 19–24) attribute an important role to recycling of organic nitrogen from expanded leaves to growing regions of the field pea.

The nitrogen economy of nonleguminous plants, however, differs from that of the field pea (25, 30) and has been far less thoroughly investigated. Amino acids labeled with ^{14}C have been applied to leaves of certain species and radioactivity detected in other parts of the plant, but the criteria for translocation of the applied substance have not always been rigorous. In some cases (12) no attempt was made to identify the radioactive compound(s) exported from the leaf; in others the identification was not unequivocal (32). In some cases synthesis of amino acids *in situ* was not excluded. This might account for their presence, especially in long term experiments (5, 7, 11, 32), but also in brief ones (15), since Turkina (29) showed that isolated conducting strands can synthesize amino acids from sucrose. Eschrich and Kating (7) applied $NaH^{14}CO_3$ to a mature leaf of *Cucurbita* and 16 hr later found several compounds, including amino acids, labeled in the hypocotyl tissue, but only sucrose and raffinose in the phloem sap exuding from the cut base of the hypocotyl.

Radioactivity is not necessarily translocated in the compound applied. When Clor (5) applied ^{14}C-urea to cotyledons of cotton, the labeled translocate in the light was sucrose, and in the dark no ^{14}C was exported. When Stewart and Beevers (26) applied ^{14}C-amino acids to the endosperm of castor bean seedlings, glutamate, aspartate, and alanine were metabolized in the endosperm and ^{14}C-sucrose transported in the phloem to the hypocotyl. Valine was transported unchanged across the cotyledons. Hofstra and Nelson (9) found that aspartate, a major initial product of photosynthesis of $^{14}CO_2$ in corn leaves, was not translocated. It was metabolized in the leaf, and only labeled sucrose was exported. This contrasts with the export of photosynthetically produced serine by soybean leaves (4, 17). An incontrovertible demonstration of translocation of labeled amino acids from mature leaves of nonleguminous plants seems to be lacking.

There appears to be no doubt that amino acids can move in the phloem. They have been identified in the phloem sap of a number of species (16, 31, 33, 34). This does not necessarily mean that they enter from leaves. Possible alternative sources are adjacent stem tissues (25) or roots (1, 10, 11, 13). Characteristically the amino acid content of phloem sap is very low in summer when the mature leaves are exporting carbohydrate abundantly, and it rises markedly in the autumn when the leaves are senescing. The general assumption is that this depends on an increased supply of free amino acids in the leaves when massive protein breakdown occurs.

The present investigation analyzes translocation of ^{14}C from nearly expanded, mature, and senescing sunflower leaves supplied briefly with $^{14}CO_2$, in which amino acids were lightly labeled. This is compared with translocation from mature leaves supplied continuously with ^{14}C-alanine for 4 hr, in which amino acids were heavily labeled.

MATERIALS AND METHODS

Plant Material. Sunflower (*Helianthus annuus* L. cv Commander) plants were grown in soil in a growth cabinet under a

[1] This work was supported in part by the National Research Council of Canada.

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14-hr day at 20,000 lux. On all experimental plants ^{14}C was applied to one leaf at the node above the primary leaves.

For $^{14}CO_2$ administration at different ages four plants each were used at 20, 30, 40, and 50 days, and two at 60 days from planting. The treated leaves at 20 days were at least three-quarters expanded, at 30 and 40 days fully expanded and green. At 50 days signs of chlorophyll breakdown were just beginning. At 60 days the leaves were predominantly yellow, but still capable of assimilating CO_2. Additional plants used for application of $^{14}CO_2$ and of ^{14}C-alanine were 30 and 35 days old, respectively.

Administration of $^{14}CO_2$. $^{14}CO_2$ was administered under fluorescent light at 27,000 lux and 23 C (3). One leaf blade on each plant was enclosed in a glass cylinder fitted with a $^{14}CO_2$ generator and a small Geiger-Müller tube. The leaf was sealed in a rubber stopper at the upper end of the petiole with a mixture of beeswax and Lubriseal, with only the blade inside the feeding chamber. $^{14}CO_2$ (171 μc except 342 μc for a yellowing leaf at 60 days) was released from $Na_2{}^{14}CO_3$ (17.1 mc/mmole) in the generator and transferred directly to the leaf chamber, and its absorption was monitored. The initial concentration of total CO_2 was close to atmospheric.

After 15 min the plants were cut into segments for analysis. These were frozen in liquid nitrogen and stored at −20 C until they could be analyzed, within a week.

In addition to the plants used in the age series, sets of four 30-day plants similarly treated with $^{14}CO_2$ for 15 min were left, under 27,000 lux, exposed to normal air under a fumehood for a further 15, 45, or 225 min before analysis.

Administration of ^{14}C-Alanine. For ^{14}C-alanine administration five plants were used in a first experiment and six in a second, at 35 days from planting. These possessed fully expanded typical leaves at the node above the primary leaves, and several partly expanded leaves above these. Any remaining cotyledons and primary leaves, some of which were senescent, were removed before the application of ^{14}C-alanine to one mature leaf at the node above the primary leaf. Uniformly labeled L-alanine (New England Nuclear Corp.) containing 12.5 μc of ^{14}C either in 50 μl (exepriment 1) or in 25 μl (experiment 2) of 0.1% Tween 20 solution was dispensed in each of two wells made from rings of Tygon tubing (i.d. 9.5 mm) and covered with glass coverslips (18). The wells were placed between main veins 2 cm from the base of the blade, sealed on with Lubriseal. The plants remained under fluorescent lights (27,000 lux) in moving air under a fumehood for 0.25, 0.5, 1, 2, 3, or 4 hr. Then radioactivity was recovered from the wells and from segments of the plants. The treated leaf was divided into four portions: the tissue immediately under the wells, the blade apical to the wells, that basal to the wells, and the petiole. These were cut into boiling 80% ethanol for analysis. In the second experiment the remainder of each plant was also cut into segments and analyzed.

Determination of Radioactivity in Ethanol Extracts. To obtain total ethanol-soluble activity, tissues were extracted four times in boiling 80% (v/v) ethanol, and aliquots of the combined extracts were counted in a thin window proportional gas flow counter. When separation into components was required, extracts were passed through Dowex 50 cation exchange resin to separate sugars and organic acids from amino acids. Aliquots of the separated fractions were counted, and the re-

Table I. *Distribution of Radioactivity in Ethanol-soluble Compounds in the Leaf Blade and Petiole after 15-min Assimilation of $^{14}CO_2$ by the Blade*

	Age of Plant in Days, and Time Lapse after Adding $^{14}CO_2$							
	20 15 min	30 15 min	40 15 min	50 15 min	60 15 min	30 30 min	30 1 hr	30 4 hr
	% of ethanol-soluble activity							
In blade								
Sucrose	73.0	70.9	76.4	62.0	78.4	27.1	10.0	0.6
Glucose + fructose	3.9	2.9	5.8	18.8	3.9	41.9	25.4	39.1
Other sugars	1.6	2.5	2.8	2.6	5.1	7.0	14.6	11.5
Malate	4.7	3.1	2.2	5.7	2.6	10.7	22.7	12.1
Other organic acids	7.5	6.7	6.4	9.4	3.6	11.2	23.3	35.4
Alanine	1.9	1.1	1.8	0.3	2.7	0.5	0.4	0.1
Glycine + serine	5.8	11.3	4.2	0.9	2.0	0.9	0.8	0
Glutamate	0.4	0.2	0.1	0.1	1.1	0.1	0.6	0.3
Other amino acids	1.3	1.3	0.3	0.2	0.6	0.8	1.5	0.9
In petiole								
Sucrose	98.7	94.3	96.9	80.6	23.4	18.7	32.1	0.6
Glucose + fructose	1.2	5.7	3.1	12.9	13.5	80.3	56.9	86.4
Other sugars	0	0	0	6.5[1]	0	0	2.1[2]	1.9[2]
Malate	0	0	0	0	15.2	0.4	1.6	1.1
Other organic acids	0	0	0	0	3.1	0.6	6.0	8.8
Alanine	0	0	0	0	22.1	0	0	0.2
Glycine + serine	0	0	0	0	13.2	0	0.7	0.2
Glutamate	0	0	0	0	5.2	0	0.2	0.2
Other amino acids	0	0	0	0	4.4	0	0.6	0.9
	ethanol-soluble activity, nc							
In blade	36,310	34,052	42,250	27,159	31,165	24,062	29,553	14,435
In petiole	1,625	1,732	103	570	89	9,585	2,903	1,986

[1] Fructosyl-sucrose.
[2] Raffinose.

mainder of the fraction was chromatographed two-dimensionally on Whatman 3MM paper using *n*-butanol-acetic acid-water (12:3:5 v/v/v) and 80% (v/v) phenol. Amino acids were located and identified by spraying with 0.2% (w/v) ninhydrin in 95% ethanol, organic acids with 0.04% (w/v) bromocresol green in ethanol at pH 7, and sugars with a 3% (w/v) solution of *para*-anisidine hydrochloride in *n*-butanol. Individual labeled compounds were identified by eluting and cochromatographing with authentic specimens. The distribution of ^{14}C in ethanol-soluble compounds was determined by exposing chromatograms to Kodak no-screen x-ray film to locate radioactive spots, excising corresponding areas of the chromatograms, and counting both sides of the paper disks.

Determination of Radioactivity in Ethanol-insoluble Fraction. When $^{14}CO_2$ was supplied and in the first experiment with ^{14}C-alanine, activity in the insoluble fractions was determined by wet combustion of residues according to the Van Slyke-Folch method, and counting the activity in the $^{14}CO_2$ evolved on a Dynacon. In the second experiment with ^{14}C-alanine, insoluble residue from leaf tissue under the wells was hydrolyzed in 6 N HCl for 24 hr at 100 C, and the hydrolysate was desalted with Dowex 50, then analyzed in the same manner as an ethanol extract.

RESULTS

When $^{14}CO_2$ was assimilated by the leaf blade, ^{14}C was detectable in most parts of the plant within 15 min, the amount exported declining with age (3). In the older plants about 90% of the ^{14}C outside the treated blade was still in the transmitting petiole, in the youngest about 50%. In the 30-day plants 3.5% of the ^{14}C was outside the treated blade after 15 min, and 46% after 4 hr when it was well distributed through the plant. At all ages some ^{14}C was in the insoluble fraction. In the treated blade this amounted to 40 to 60% of the total, in the petiole usually about 1% at 15 min, increasing to 40% after 4 hr in the 30-day plants. In these plants the insoluble ^{14}C reached more than 60% of the total in the stem tip and roots at 4 hr (3).

For a comparison of translocation in plants of all ages only the treated blade and its petiole were always sufficiently labeled to permit chromatographic analysis of activity in individual compounds. The activity in components of the ethanol extracts of these organs is shown in Table I. The total ethanol-soluble activity shown is the average value for four plants. The percentage activity in components usually derives from two or three plants, occasionally from one. Only conspicuous differences are regarded as important. In the leaf blade at 15 min sucrose carried most of the ^{14}C in the soluble fraction, but both organic and amino acids were lightly labeled in plants of all ages. In the petiole at the same time no label was detectable in soluble substances other than sugars in 20- to 50-day-old plants. In the petiole of senescent leaves at 60 days amino acids contained more ^{14}C than did sugars. In the 30-day plants no amino acids appeared in the petiole until 1 hr had elapsed, and after 4 hr they accounted for 1.5% of the total ethanol-soluble activity, as compared with 45% in the 60-day petiole at 15 min. During the 4-hr interval metabolism was obviously occurring in the petiole. Labeled sucrose was extensively hydrolyzed, and organic acids and insoluble compounds also became labeled.

The distribution of ^{14}C in other parts of young and mature plants is shown in Table II for those parts that contained enough ^{14}C for chromatographic analysis. After 15 min sucrose was virtually the only substance labeled, especially in the regions most recently receiving translocate. In time, labeled sucrose disappeared. Most of the ^{14}C remained in sugars, chiefly hexoses, but also in fructosans, especially in the middle and lower portions of the stem. Organic and amino acids also acquired label, and after 1 or 4 hr amino acids contained relatively more ^{14}C in all other parts of the plant than in the transmitting petiole (Table I). The pattern clearly suggests that sucrose was translocated and then metabolized.

Table II. *Distribution of Radioactivity in Ethanol-soluble Compounds in the Plant after 15-min Assimilation of $^{14}CO_2$ by a Leaf Blade*

	Age of Plant in Days, and Time Lapse after Adding $^{14}CO_2$				
	20 15 min	30 15 min	30 30 min	30 1 hr	30 4 hr
	% of ethanol-soluble activity				
Shoot above fed leaf					
Sucrose	98.7	100	69.1	63.5	0.7
Total sugars	100	100	99.1	86.8	75.9
Organic acids	0	0	0.2	7.4	18.7
Amino acids	0	0	0.8	5.8	5.4
Shoot below fed leaf					
Upper					
Sucrose	98.7	97.6	75.1	63.5	1.3
Total sugars	99.4	100	99.0	87.0	81.8
Organic acids	0.2	0	0.6	10.1	15.1
Amino acids	0.4	0	0.4	2.9	3.1
Middle					
Sucrose	98.7	98.7	76.5	65.8	5.2
Total sugars	99.8	100	99.4	92.5	91.1
Organic acids	0.1	0	0.2	5.6	6.9
Amino acids	0.1	0	0.4	2.1	2.0
Lower					
Sucrose	98.5	98.5	85.6	75.2	3.7
Total sugars	99.9	100	99.9	93.3	93.3
Organic acids	0	0	0	4.6	4.6
Amino acids	0.1		0.1	2.1	2.1
Roots					
Sucrose	100	100	77.4	63.6	6.2
Total sugars	100	100	99.2	93.9	89.9
Organic acids	0	0	0	3.0	7.6
Amino acids	0	0	0.9	3.1	2.6
	ethanol-soluble activity, nc				
Shoot above fed leaf	13	23	1917	5271	4515
Shoot below fed leaf					
Upper	415	434	6330	6542	3812
Middle	860	114	4389	4971	4469
Lower	233	6	1865	4497	3143
Roots	10	2	1065	7777	3835

The data presented indicate that up to 40 days the mature leaf blade exported only sucrose. At the stage when chlorophyll breakdown was beginning (50 days) hexoses may also be exported to a small extent, although the increased content of labeled hexoses in the petiole could alternatively result from hydrolysis of sucrose *in situ*. The predominantly yellow leaves (60 days) apparently also exported malic acid and a variety of amino acids, among which alanine carried about half the label.

In the foregoing experiments amino acids were only lightly labeled in the leaf blade. The experiments in which ^{14}C-alanine was administered to mature leaves provided contrasting conditions where high concentrations of labeled amino acids were maintained in the absorbing tissue while translocation was followed for 4 hr. The numerical data pre-

Table III. *Distribution of Radioactivity in Sunflower Plants Absorbing ^{14}C-Alanine from Wells Attached to a Mature Leaf*

	Radioactivity[1] after Absorption for:					
	0.25 hr	0.5 hr	1 hr	2 hr	3 hr	4 hr
	nc					
Under wells						
Soluble	507.6	1,025.3	1,774.8	2,431.9	2,475.3	3,421.4
Insoluble	124.0	527.0	171.0	608.0	495.0	1,665.0
Total	631.6	1,552.3	1,945.8	3,039.9	2,970.3	5,086.4
Fed leaf outside wells						
Blade, apical	0.3	6.0	4.9	10.2	17.8	80.1
Blade, basal	1.1	28.0	31.1	69.7	52.9	113.6
Petiole	0.7	24.7	28.1	44.2	35.1	48.9
Total soluble	2.1	58.7	64.1	124.1	105.8	242.6
Rest of plant						
Above fed leaf	0	2.6	11.0	20.9	37.0	65.2
Below fed leaf	0	21.7	49.5	137.4	223.8	381.9
Total soluble	0	24.3	60.5	158.3	260.8	447.1
Unabsorbed	24,200	22,000	19,200	13,400	3,700	2,000
Percentage recorded[2]	99.5	95	85	67	28	31

[1] Activity in ethanol-soluble compounds only, except under the supply wells.

[2] Percentage of ^{14}C offered (25,000 nc) that was measured (excluding activity in insoluble compounds outside the supply areas and $^{14}CO_2$ lost in respiration).

sented are from the second experiment. Those of the first experiment were entirely consistent with these.

Absorption of the alanine offered was approximately linear with time and was nearly complete (92%) within 4 hr. Radioactivity in ethanol-soluble compounds in various tissues after different time intervals is shown in Table III. Activity in insoluble compounds is recorded only for tissue under the wells. In other parts of the leaf insoluble activity did not exceed 5% of the total (experiment 1). In other parts of the plant, especially the roots and stem apex, it would be higher, but was not measured, since the emphasis was on the components carrying ^{14}C in transit. The radioactivity measured after 0.25 hr of absorption of ^{14}C-alanine accounted for 99.5% of that presented; after 0.5 hr, 95%. As time went on, less was accounted for until, at 4 hr, the measured activity represented only about 30% of that presented. The difference would be the result of accumulation of insoluble compounds in various parts of the plant, and the loss of $^{14}CO_2$ by respiration. Both would naturally increase with time.

During the first 15 min very little ^{14}C was exported from the absorbing tissue, and none passed beyond the petiole of the fed leaf. Thereafter radioactivity was found in all parts of the plant, in increasing amounts as time went on. Distribution was like that from a corresponding leaf of a 30-day-old plant assimilating $^{14}CO_2$.

The amount transported was small in comparison with that from a whole leaf exposed to $^{14}CO_2$. This may be attributed partly to the fact that the dosage of ^{14}C was much smaller and was absorbed gradually over a 4-hr period and partly to the restricted area of application 1.4 cm^2 as compared with 66 cm^2 for the whole leaf. In experiment 2, 3.1% of the ^{14}C absorbed was found in soluble compounds outside the treated leaf after 4 hr. This corresponds to the finding of Halevy *et al.* (8) that 3.4% of the soluble ^{14}C was outside the leaf 4 hr after similar application of labeled sucrose to a bean leaf held in the light. Nelson and Gorham (18) found that, of the ^{14}C absorbed as sucrose by a soybean leaf from a 1-cm well, only 1% was exported during 14 hr in light, while application of $^{14}CO_2$ to a whole leaf resulted in export of 15% of the ^{14}C in 3 hr.

Table IV shows the concentration of total soluble ^{14}C in different portions of the leaf as nc/g fresh weight, which would be approximately equivalent to nc/cm^3. For 3 hr it was 6 to 10 times as high in the basal as in the apical portion, and concentration in the petiole was nearly the same as in the base of the blade. This pattern indicates that ^{14}C was being exported from the absorbing region, predominantly basally, and was not merely being incorporated by photosynthesis of escaped $^{14}CO_2$. This was borne out by the distribution in the rest of the plant, with accumulation in bud and roots, and absence from expanded leaves.

It cannot, however, be assumed that the ^{14}C was translocated as alanine. Table V presents evidence that it was not. Although labeled alanine was always plentiful in the absorbing tissues, only minute amounts appeared in the basal portion of the leaf blade outside the areas under the wells, and almost none in the petiole at any time during the 4-hr experimental period. The predominantly labeled substance in the transmitting tissue, both basal leaf and petiole, was sucrose. Very small quantities of ^{14}C in these tissues appeared in other sugars, amino acids, and organic acids, and in ethanol-insoluble compounds. The radioactivity in alanine did not increase with time, while labeled sucrose was increasing 100-fold.

The radioactivity in alanine in the absorbing tissue remained nearly constant as absorption continued, but the alanine was readily metabolized and many substances acquired label. These included sucrose and other sugars, mainly hexoses, also organic acids and a number of amino acids. Of the amino acids, glutamic carried most label, but aspartic acid, serine, glutamine, and valine were also labeled in the absorbing tissue. In the basal portion of the leaf and the petiole no labeled glutamine or valine was detected at any time and very little of the other amino acids, including alanine. The activity of alanine in the absorbing tissue was at all times higher than that of hexoses, but in the petiole it was consistently lower, and the total activity of all amino acids was of the same magnitude as that of hexoses.

Table IV. *Concentration of Total ^{14}C in Ethanol-soluble Compounds in Different Parts of a Leaf Absorbing ^{14}C-Alanine from Wells Attached to the Surface*

	Absorption Time, hr					
	0.25	0.5	1	2	3	4
	radioactivity, nc/g fresh wt					
Under wells	4,615	9,406	13,245	20,266	16,954	32,900
Blade, apical	0.6	15	5.8	17	14	66
Blade, basal	3.4	126	56	162	83	167
Petiole	2.6	142	42	129	54	153
	ratios of activity in different parts of the leaf					
Basal/apical	5.7	8.4	9.6	9.5	5.9	2.5
Petiole/apical	4.3	9.5	7.2	7.6	3.9	2.3
Petiole/basal	0.8	1.1	0.8	0.8	0.7	0.9
Under wells/apical	7,360	627	2,280	1,192	1,200	495
Under wells/basal	1,360	75	237	125	209	197
Under wells/petiole	1,775	66	300	157	318	215

Table V. *Distribution of Radioactivity in Ethanol Extracts of Leaf Tissues during Absorption of ^{14}C-Alanine from Wells Attached to the Surface*

	Radioactivity after Absorption for:					
	0.25 hr	0.5 hr	1 hr	2 hr	3 hr	4 hr
	nc					
Under wells						
Alanine	360.4	410.1	372.7	364.8	346.5	205.3
Glutamic acid	14.8	138.4	176.5	395.1	247.7	339.8
Other amino acids	15.7	117.7	142.4	213.6	173.2	139.2
Sucrose	86.3	164.1	621.2	686.6	915.9	958.0
Oligosaccharides[1]	0	0	24.9	28.6	81.3	60.6
Hexoses	15.3	10.2	63.8	87.5	67.2	178.8
Organic acids	15.2	184.6	372.7	656.6	643.6	1505.4
Blade, basal						
Alanine	0.2	0.3	1.5	0.7	0.5	1.1
Glutamic acid	0.2	0.6	0.7	1.5	0.9	2.4
Other amino acids	0	0.5	0	0.6	0.7	2.1
Sucrose	0.7	23.8	24.9	57.2	30.2	71.6
Oligosaccharides[1]	0	0.9	0.5	2.2	0.8	4.1
Hexoses	0	1.1	3.2	2.6	1.8	8.3
Organic acids	0	0.8	0.6	4.9	18.0	23.9
Petiole						
Alanine	0.2	0.5	0	Trace	Trace	0.2
Glutamic acid	0.15	2.4	0.5	1.2	0.8	2.0
Other amino acids	0	0.6	0.3	1.0	0.9	2.1
Sucrose	0.35	15.3	25.3	36.2	29.5	36.2
Oligosaccharides[1]	0	0	0.8	2.8	1.7	0.7
Hexoses	0	1.0	1.2	1.8	1.4	3.7
Organic acids	0	4.9	Trace	1.3	0.7	3.9

[1] Other than sucrose.

The origin of small amounts of labeled metabolites in the petiole cannot be determined from the present data, since the whole tissue was extracted. The presence of ethanol-insoluble compounds indicates that translocate was metabolized in the petiole. Hexoses are presumed to derive from translocated sucrose, and the amino acids may well have arisen in the same way. There is no positive evidence that any amino acid entered the sieve tubes. The possibility cannot be excluded that minute amounts of alanine and glutamic acid were transported *via* the phloem, but there was no major export. Much of the ^{14}C absorbed as alanine found its way into sucrose, and this was exported.

DISCUSSION

The pathway by which ^{14}C entered sucrose from alanine is of interest in relation to leaf metabolism, though not directly relevant to the observations made here. The plants were lighted throughout the experimental period, and it is possible that $^{14}CO_2$ produced in respiration was reassimilated in photosyn-

thesis. If so, it must have been largely $^{14}CO_2$ that remained within the absorbing region and never escaped into the surrounding atmosphere, since the concentration of radioactivity in the surrounding leaf blade was from 2 to more than 3 orders of magnitude lower than under the supply wells (Table IV). By whatever metabolic pathway, sucrose became heavily labeled in the absorbing tissue, and sucrose, not alanine, was the major, if not the only substance exported from this region.

The data presented make it clear that a mechanism for selection of translocate exists within the leaf. The critical step may well be entry into the sieve tube, and the determining factor the existence or operation of a mechanism to accomplish this, as Kursanov (14) maintains.

When sunflower plants of different ages were supplied with $^{14}CO_2$, yellowing leaves exported labeled amino acids, but green leaves under the same external conditions did not. Evidently this was not merely because the supply of labeled amino acids was low in green leaves. In the first place, it was also low in yellowing leaves, which exported amino acids. In the second place, a very large increase in supply in green leaf tissue provided with ^{14}C-alanine did not lead to corresponding export. This suggests, rather, a change in the mechanism controlling entry into the phloem in senescing leaves, whether this is metabolic in nature (14) or resides in the properties of the limiting membrane (28).

It is recognized that the data presented here pertain to one set of experimental conditions. Possibly under other conditions amino acids are freely exported by mature leaves. It remains to be explicitly demonstrated whether this does occur and, if it does, what the requisite conditions are.

Wallace and Pate (30) devised a scheme for the economy of nitrogen in *Xanthium* that included recycling of organic nitrogen from mature leaves *via* the phloem. They found no evidence for export of amino acids to young growing organs from expanded leaves after 1 hr of assimilation of $^{14}CO_2$. They inferred, however, that organic nitrogen is exported because the content of protein and free amino acids in the leaves does not increase after they are fully expanded. In *Xanthium* the relevance of this interpretation is complicated by the fact that, although nitrate is immediately reduced on entering the mesophyll, it remains unchanged in the veins. Their data show (30, Table 2) that 98 to 99% of the soluble nitrogen content of middle and old leaves was in the form of nitrate.

Recycling of organic nitrogen from mature leaves of the field pea apparently plays an important part in the nitrogen economy of this plant (19, 21, 24), but it is not impossible that in some species it is of little or no significance, at least in the vegetative phase. Nonleguminous plants have a different nitrogen economy, and none has been so thoroughly investigated as the field pea.

Pate, Wallace, and van Die (25) reported that in the field pea the bulk of organic nitrogen from the roots was delivered to the older leaves, and Pate, Walker, and Wallace (24) showed that later transfer to upper parts of the shoot may occur. In cocklebur, tomato, and balsam, however, the larger proportion of nitrogen, organic and inorganic, was delivered directly to younger parts of the shoot *via* the xylem.

Joy (11) found that all the mature leaves could be removed from sugar beet plants without reducing the growth or the nitrogen content of young, expanding leaves; but removal of fibrous roots prevented growth. Amino acids were rapidly synthesized (5 min) from ^{14}C-sucrose applied directly to young leaves, and the labeling pattern of protein suggested that glutamic acid or glutamine derived from nitrate reduction was supplied by the roots, as well as nitrate. He concluded that mature leaves contributed little or no nitrogen.

We have little definitive information about the movements of organic nitrogen in most plant species. While the expectation may be that organic nitrogen is recycled from mature leaves to support growth, this cannot be assumed for all species without explicit demonstration. Further investigation, using isotopic tracers with nonleguminous plants under a variety of conditions, would be useful.

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